

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
1 November 2001 (01.11.2001)

PCT

(10) International Publication Number  
**WO 01/80639 A2**

(51) International Patent Classification<sup>7</sup>: **A01N 37/46**,  
63/00, 63/02

(21) International Application Number: PCT/US01/12468

(22) International Filing Date: 17 April 2001 (17.04.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/198,359 19 April 2000 (19.04.2000) -US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,  
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— without international search report and to be republished  
upon receipt of that report

*For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.*

(54) Title: TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE RESPONSE ELICITOR TO INHIBIT  
POSTHARVEST DISEASE OR DESICCATION

(57) Abstract: The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable, either by treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation, or by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant. Also disclosed are DNA constructs and expression systems, host cells, and transgenic plants containing the DNA construct.

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**TREATMENT OF FRUITS OR VEGETABLES WITH  
HYPERSENSITIVE RESPONSE ELICITOR TO INHIBIT  
POSTHARVEST DISEASE OR DESICCATION**

5                   This application claims benefit of U.S. Provisional Patent Application  
Serial No. 60/198,359, filed April 19, 2000, which is hereby incorporated by  
reference in its entirety.

**FIELD OF THE INVENTION**

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The present invention relates to methods of treating fruits or vegetables  
to inhibit postharvest diseases and/or desiccation of harvested fruits or vegetables.

**BACKGROUND OF THE INVENTION**

15

Postharvest diseases are often extensions of disease occurring in the  
field or orchard. Brown rot of stone fruits (*Monilinia fructicola* (Wint.) Honey), for  
example, may cause blossom and twig blighting in the orchard. Infections in the  
orchard may not be visible at harvest if fruits are not refrigerated. *Colletotrichum*  
20 *gloeosporioides* (Penz.) Arx may attack blossoms or leaves and young fruit of citrus,  
avocados, mangos, papayas, and a wide range of other tropical and subtropical  
species; infections in developing fruit are usually latent, and rot lesions appear only at  
the onset of fruit ripening. *Pezicula malicorticis* (Jacks.) Nannfld. causes cankers of  
limbs of apples and pears; infections in developing fruit are latent, and active rotting  
25 usually commences only after the fruit has spent several months in storage and  
proceeds during -1°C storage because the organism is able to grow at very low  
temperatures. These fungi used as examples are able to penetrate the cuticle and  
epidermis of the fruit.

Whether capable of being penetrated directly or not, wounds are often  
30 the usual means by which the fungus enters fruit. Cuts, punctures, bruises, and  
abrasions cannot be avoided completely during harvest and handling. If the cuticle  
and epidermis are broken, spores find nutrients and humidity in fresh wounds ideal for  
spore germination and colonization. Separation of fruits from the parent plant at  
harvest creates an unavoidable wound that encourages stem-end rots.

Rots developing at the blossom end usually involve prior colonization of floral parts. For example, *Botrytis* blossom-end rot (*B. cinerea*) sometimes occurs in Bartlett pears after a month or two in storage at -1°C. Initiation of rot in fruit flesh is associated with old styles and stamens retained within the fruit. Floral infections occur in the senescing floral parts at the end of blossoming. Mostly these floral parts are invaded by *Alternaria* spp. and common saprophytic fungi, but *B. cinerea* also is found occasionally. Not all fruits having *B. cinerea*-invaded floral parts rot in storage, but a significant percentage do. By contrast, test fruits remain free from *Botrytis* blossom-end rot if the old floral parts of developing fruits are free from *B. cinerea*. Rotting of fruits in storage is greatly reduced by a single orchard spray with a fungicide at the end of blossoming.

Contact infection, by which mycelia grow from a rotting fruit to contact and penetrate nearby fruit, is an especially serious aspect of some very common postharvest pathogens. The ever-enlarging "nest" of rotting fruit tied together by fungus mycelia will involve all fruit in a container, if given sufficient time.

Disease or threat of disease dictates in large measure the manner in which perishable fruits are handled. In recent decades, fruits have been shipped to increasingly greater distances from points of production. Exploitation of these distant markets, however, may offer large economic benefits only if the life of the commodity is stretched to its limit. Diseases and disorders ordinarily manageable during handling and transcontinental transit and marketing may be excessive when transoceanic marine transport of longer duration is involved. Similarly, the extension of marketing periods by storing fruits until they near the end of their physiological life may cause additional disease problems. Losses are especially serious if they occur in market areas, because the costs of sorting, packaging, cooling, storage, and transportation, which may greatly exceed production costs, have already been incurred. Of even greater long-term importance may be an impaired reputation leading to reduced future sales.

Postharvest diseases of fruit cause 15 to 25% losses yearly in the fruit industry worldwide and much of this is due to rot caused by microorganisms. Fungicides, which have been the primary means of controlling postharvest diseases, have come under scrutiny as posing potential oncogenic risks when applied to

processed foods. Thus, research efforts have been intensified to develop biological control procedures for postharvest diseases of fruits and vegetables that pose less risk to human health and the environment.

Considerable attention has been placed on assessing the use of antagonistic microorganisms as a viable alternative to the use of synthetic fungicides. Two basic approaches are available for using antagonistic microorganisms to control postharvest diseases. Naturally occurring antagonists that already exist on fruit and vegetable surfaces have been shown to control several rot pathogens on diverse commodities. Alternatively, artificially introduced antagonists have been shown to be effective in biologically controlling postharvest pathogens.

Since 1983, an explosion of research has occurred in the area of biological control of postharvest diseases by artificially introduced antagonists, mostly on fruit diseases (Janisiewicz, "Biological Control of Diseases of Fruit," In Biocontrol of Plant Diseases II, Mukergie et al. (ed.), CRC Press, Boca Raton, pp. 153-165 (1988) and Wilson et al., "Potential for Biological Control of Postharvest Plant Diseases," Plant Disease 69:375-378 (1985)). For example, rot on apples was controlled with yeast (Wisniewski et al., "Biological Control of Postharvest Diseases of Fruit: Inhibition of *Botrytis* Rot on Apples by an Antagonistic Yeast," Proc. Electron Microsc. Soc. Am. 46:290-91 (1988)), while brown rot in apricots was controlled with *Bacillus subtilis* (Pusey et al., "Postharvest Biological Control of Stone Fruit Brown Rot by *Bacillus subtilis*," Plant Dis. 68:753-56 (1984)). Mold incidence was reduced from 35% to 8% in lemon peel by a species of *Trichoderma* (De Matos, "Chemical and Microbiological Factors Influencing the Infection of Lemons by *Geotrichum candidum* and *Penicillium digitatum*," Ph.D. dissertation, University of California, Riverside, 106 pp. (1983)). Biocontrol of citrus rot pathogens was demonstrated with *Bacillus subtilis* (Singh et al., "*Bacillus subtilis* as a Control Agent Against Fungal Pathogens of Citrus Fruit," Trans. Br. Mycol. Soc. 83:487-90 (1984)). Such antagonists have various modes of action: antibiosis or competition for nutrients and space or both, induction of resistance in the host tissue, and direct interaction with the pathogen (Wilson et al., "Biological Control of Postharvest Diseases of Fruits and Vegetables: An Emerging Technology," Annu. Rev. Phytopathol. 27:425-441 (1989)).

While treatment with antagonistic bacterial or fungal species may be, at least to some extent, effective in controlling postharvest diseases, there are a number of factors which must be considered before this approach is used in commercial applications. First, the antagonists must be grown and maintained for use  
5 in treatments. This may result in significant expense and regulatory burdens depending on when and how frequently such antagonists would be applied. Also, it is questionable whether growers would want to maintain bioreactors for growing and propagating particular antagonist strains. Second, the efficacy of those antagonists may depend on storage conditions during shipment of harvested fruit. Some  
10 antagonists may not be able to tolerate variations in conditions during shipment, thereby allowing the pathogens to overcome any inhibitory effects of the antagonists. Given the above problems, it is not surprising that few of the antagonists reported to control plant pathogens have been successfully transferred from the laboratory into the field or postharvest environment.

15 Thus, there still exists a need to provide an effective, commercially viable method for treating fruits and vegetables to control postharvest diseases which avoids entirely or otherwise significantly reduces the need for fungicide treatments. In particular, it would be desirable to provide an effective, practicable treatment which presents little or no harm to humans or the environment.

20 The present invention is directed to overcoming these and other deficiencies in the art.

### SUMMARY OF THE INVENTION

25 The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

30 A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

Another aspect of the present invention relates to a DNA construct that includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule, wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation. Also disclosed are expression systems, host cells, and transgenic plants which contain a heterologous DNA construct of the present invention.

By the present invention, the hypersensitive response elicitor protein or polypeptide can be used to inhibit or otherwise control postharvest diseases (i.e., caused by pathogens) in fruits or vegetables. Likewise, such treatment can also inhibit postharvest desiccation of treated fruits or vegetables. In achieving these objectives, the present invention enables produce growers, warehouse packers, shippers, and suppliers to process, handle, and store fruits and vegetables with reduced losses caused by postharvest disease and desiccation. As a result, the cost of bringing fruits and vegetables from the field to the consumer can be reduced. Importantly, the quality of the treated fruits or vegetables is improved.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.

A further aspect of the present invention relates to another method of inhibiting postharvest disease or desiccation in a fruit or vegetable. This method is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to inhibit a postharvest disease or desiccation in a fruit or vegetable harvested from the transgenic plant.

For use in accordance with these methods, suitable hypersensitive response elicitor proteins or polypeptides are those derived from a wide variety of bacterial and fungal pathogens, preferably bacterial pathogens.

Exemplary hypersensitive response elicitor proteins and polypeptides from bacterial sources include, without limitation, the hypersensitive response elicitors derived from *Erwinia* species (e.g., *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, etc.), *Pseudomonas* species (e.g., *Pseudomonas syringae*, *Pseudomonas solanacearum*, etc.), and *Xanthomonas* species (e.g., *Xanthomonas campestris*). In addition to hypersensitive response elicitors from these Gram-negative bacteria, it is possible to use elicitors derived from Gram-positive bacteria. One example is the hypersensitive response elicitor derived from *Clavibacter michiganensis* subsp. *sepedonicus*.

Exemplary hypersensitive response elicitor proteins or polypeptides from fungal sources include, without limitation, the hypersensitive response elicitors (i.e., elicitors) from various *Phytophthora* species (e.g., *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, *Phytophthora citrophthora*, etc.).

Preferably, the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*, *Erwinia amylovora*, *Pseudomonas syringae*, or *Pseudomonas solanacearum*.

A hypersensitive response elicitor protein or polypeptide from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser	1	5	10	15
Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser	20	25	30	
Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr	35	40	45	
Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu	50	55	60	
Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser	65	70	75	80



	Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys	
					85					90					95		
	Ser	Gly	Gly	Asp	Ala	Leu	Ser	Lys	Met	Phe	Asp	Lys	Ala	Leu	Asp	Asp	
				100					105					110			
5	Leu	Leu	Gly	His	Asp	Thr	Val	Thr	Lys	Leu	Thr	Asn	Gln	Ser	Asn	Gln	
			115					120					125				
	Leu	Ala	Asn	Ser	Met	Leu	Asn	Ala	Ser	Gln	Met	Thr	Gln	Gly	Asn	Met	
		130					135					140					
10	Asn	Ala	Phe	Gly	Ser	Gly	Val	Asn	Asn	Ala	Leu	Ser	Ser	Ile	Leu	Gly	
		145				150					155					160	
	Asn	Gly	Leu	Gly	Gln	Ser	Met	Ser	Gly	Phe	Ser	Gln	Pro	Ser	Leu	Gly	
				165						170					175		
	Ala	Gly	Gly	Leu	Gln	Gly	Leu	Ser	Gly	Ala	Gly	Ala	Phe	Asn	Gln	Leu	
				180					185					190			
15	Gly	Asn	Ala	Ile	Gly	Met	Gly	Val	Gly	Gln	Asn	Ala	Ala	Leu	Ser	Ala	
		195					200					205					
	Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val	
		210					215					220					
20	Asp	Lys	Glu	Asp	Arg	Gly	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	Asp	
		225				230					235					240	
	Gln	Tyr	Pro	Glu	Ile	Phe	Gly	Lys	Pro	Glu	Tyr	Gln	Lys	Asp	Gly	Trp	
				245						250					255		
	Ser	Ser	Pro	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser	Lys	
				260					265					270			
25	Pro	Asp	Asp	Asp	Gly	Met	Thr	Gly	Ala	Ser	Met	Asp	Lys	Phe	Arg	Gln	
			275					280					285				
	Ala	Met	Gly	Met	Ile	Lys	Ser	Ala	Val	Ala	Gly	Asp	Thr	Gly	Asn	Thr	
		290					295					300					
30	Asn	Leu	Asn	Leu	Arg	Gly	Ala	Gly	Gly	Ala	Ser	Leu	Gly	Ile	Asp	Ala	
		305				310					315					320	
	Ala	Val	Val	Gly	Asp	Lys	Ile	Ala	Asn	Met	Ser	Leu	Gly	Lys	Leu	Ala	
				325						330					335		
	Asn	Ala															

35 This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. This *Erwinia chrysanthemi* hypersensitive response elicitor protein or polypeptide is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

40 cgattttacc cgggtgaacg tgctatgacc gacagcatca cggatttcga caccgttacg 60  
 gcgttttatgg ccgcgatgaa ccggcatcag gcggcgcgct ggtcgcccga atccggcgctc 120  
 gatctggtat ttcagtttgg ggacaccggg cgtgaactca tgatgcagat tcagccgggg 180  
 cagcaatatc ccggcatggt gcgcacgctg ctcgctcgctc gttatcagca ggcggcagag 240

	tgcatggct gccatctgtg cctgaacggc agcgtgtat tgatcctctg gtggccgctg	300
	ccgtcggatc ccggcagtta tccgcaggtg atcgaacgtt tgtttgaact ggcgggaatg	360
	acgttgccgt cgctatccat agcacggacg gcgcgtccgc agacagggaa cggacgcgcc	420
	cgatcattaa gataaaggcg gcttttttta ttgcaaacg gtaacgggtga ggaaccgttt	480
5	caccgtcggc gtcactcagt aacaagtatc catcatgatg cctacatcgg gatcggcgtg	540
	ggcatccgtt gcagatactt ttgcgaacac ctgacatgaa tgaggaaaacg aaattatgca	600
	aattacgatc aaagcgcaca tcggcgggtga tttgggcgtc tccgggtctgg ggctgggtgc	660
	tcagggactg aaaggactga attccgcggc ttcacgtctg ggttccagcg tggataaact	720
	gagcagcacc atcgataagt tgacctccgc gctgacttct atgatgtttg gcggcgcgct	780
10	ggcgcagggg ctgggcgccca gctcgaaggg gctggggatg agcaatcaac tgggccagtc	840
	tttcggcaat ggcgcgcagg gtgcgagcaa cctgctatcc gtaccgaaat ccggcggcga	900
	tgcggtgtca aaaatgtttg ataaagcgct ggacgatctg ctgggtcatg acaccgtgac	960
	caagctgact aaccagagca accaactggc taattcaatg ctgaacgccca gccagatgac	1020
	ccagggtaat atgaatgcgt tcggcagcgg tgtgaacaac gcactgtcgt ccattctcgg	1080
15	caacgggtctc ggccagtcga tgagtggctt ctctcagcct tctctggggg caggcggtt	1140
	gcagggcctg agcggcgcgg gtgcattcaa ccagttgggt aatgccatcg gcattggcgt	1200
	ggggcagaat gctgcgtga gtgcgttgag taacgtcagc acccacgtag acggtaacaa	1260
	ccgccacttt gtagataaag aagatcgcgg catggcgaaa gagatcggcc agtttatgga	1320
	tcagtatccg gaaatattcg gtaaaccgga ataccagaaa gatggctgga gttcgccgaa	1380
20	gacggacgac aaatcctggg ctaaagcgct gagtaaaccg gatgatgacg gtatgaccgg	1440
	cgccagcatg gacaaattcc gtcaggcgat gggatatgatc aaaagcgcgg tggcgggtga	1500
	taccggcaat accaacctga acctgcgtgg cgcgggcggg gcacgtctgg gtatcgatgc	1560
	ggctgtcgtc ggcgataaaa tagccaacat gtcgtgggt aagctggcca acgcctgata	1620
	atctgtgctg gcctgataaa gcggaaacga aaaaagagac ggggaagcct gtctcttttc	1680
25	ttattatgcg gtttatgcg ttacctggac cggttaatca tcgtcatcga tctggtacaa	1740
	acgcacattt tcccgttcat tcgcgtcgtt acgcgccaca atcgcgatgg catcttctc	1800
	gtcgtcaga ttgcgcggct gatggggaac gccgggtgga atatagagaa actcgccggc	1860
	cagatggaga cacgtctgcg ataaatctgt gccgtaacgt gtttctatcc gcccttttag	1920
	cagatagatt gcggtttcgt aatcaacatg gtaatgcgt tccgcctgtg cgcgggcgg	1980
30	gatcaccaca atattcatag aaagctgtct tgcacctacc gtatcgcggg agataccgac	2040
	aaaatagggc agtttttgcg tggatatcgt ggggtgttcc ggctgacaa tcttgagttg	2100
	gttcgtcatc atctttctcc atctggcgca cctgatcggg t	2141

The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,850,015 to Bauer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

5	Met	Ser	Leu	Asn	Thr	Ser	Gly	Leu	Gly	Ala	Ser	Thr	Met	Gln	Ile	Ser	1	5	10	15
	Ile	Gly	Gly	Ala	Gly	Gly	Asn	Asn	Gly	Leu	Leu	Gly	Thr	Ser	Arg	Gln	20	25	30	
10	Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Gly	Asn	35	40	45	
	Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met	50	55	60	
	Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Gly	Leu	Met	Gly	Gly	Gly	Leu	65	70	75	80
15	Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu	85	90	95	
	Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Asn	Thr	100	105	110	
20	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro	115	120	125	
	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser	130	135	140	
	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln	145	150	155	160
25	Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly	165	170	175	
	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu	180	185	190	
30	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly	195	200	205	
	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly	210	215	220	
	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu	225	230	235	240
35	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln	245	250	255	
	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln	260	265	270	
40	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe	275	280	285	
	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met	290	295	300	
	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro	305	310	315	320
45	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser	325	330	335	

Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn  
 340 345 350  
 Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn  
 355 360 365  
 5 Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp  
 370 375 380  
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu  
 385 390 395 400  
 Gly Ala Ala

10

15

This hypersensitive response elicitor protein or polypeptide has a molecular weight of  
 about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10  
 minutes. This hypersensitive response elicitor protein or polypeptide has substantially  
 no cysteine. The hypersensitive response elicitor protein or polypeptide derived from  
*Erwinia amylovora* is more fully described in Wei, Z-M., et al., "Harpin, Elicitor of  
 the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,"  
Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety.  
 The DNA molecule encoding this hypersensitive response elicitor protein or  
 polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

20

25

30

35

aagcttcggc atggcacgtt tgaccgttgg gtcggcaggg tacgtttgaa ttattcataa 60  
 gaggaatacgt ttatgagtct gaatacaagt gggctgggag cgtcaacgat gcaaatttct 120  
 atcggcgggtg cgggcggaaa taacgggttg ctgggtacca gtcgccagaa tgctgggttg 180  
 ggtggcaatt ctgcactggg gctgggcggc ggtaatcaaa atgataccgt caatcagctg 240  
 gctggcttac tcaccggcat gatgatgatg atgagcatga tgggcgggtg tgggctgatg 300  
 ggcgggtggct taggcgggtg cttaggtaat ggcttgggtg gctcaggtg cctgggcgaa 360  
 ggactgtcga acgcgctgaa cgatatgtta ggcggttcgc tgaacacgct gggctcgaaa 420  
 ggcggcaaca ataccacttc aacaacaaat tccccgctgg accaggcgct gggattatac 480  
 tcaacgtccc aaaacgacga ttccacctcc ggcacagatt ccacctcaga ctccagcgac 540  
 ccgatgcagc agctgctgaa gatgttcagc gagataatgc aaagcctgtt tgggtgatggg 600  
 caagatggca cccagggcag ttctctctggg ggcaagcagc cgaccgaagg cgagcagaac 660  
 gcctataaaa aaggagtcac tgatgcgctg tcgggcctga tgggtaattg tctgagccag 720  
 ctctttggca acgggggact gggaggtggt cagggcggtg atgctggcac gggctctgac 780  
 ggttcgtcgc tgggcggcaa agggctgcaa aacctgagcg ggccggtgga ctaccagcag 840  
 ttaggtaacg ccgtgggtac cggatatcgg atgaaagcgg gcattcaggc gctgaatgat 900  
 atcggtagcg acaggcacag ttcaaccctg tctttcgtca ataaaggcga tcgggcgatg 960  
 gcgaaggaaa tcggtcagtt catggaccag tatcctgagg tgtttggcaa gccgcagtac 1020  
 cagaaaggcc cgggtcagga ggtgaaaacc gatgacaaat catgggcaaa agcactgagc 1080  
 aagccagatg acgacggaat gacaccagcc agtatggagc agttcaacaa agccaagggc 1140

atgatcaaaa ggcccatggc gggtgatacc ggcaacggca acctgcaggc acgcggtgcc 1200  
 ggtggttctt cgctgggtat tgatgccatg atggccggtg atgccattaa caatatggca 1260  
 cttggcaagc tgggcgcggc ttaagctt 1288

5 The above nucleotide and amino acid sequences are disclosed are further described in U.S. Patent No. 5,849,868 to Beer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

	Met	Ser	Ile	Leu	Thr	Leu	Asn	Asn	Asn	Thr	Ser	Ser	Ser	Pro	Gly	Leu	
	1				5					10					15		
15	Phe	Gln	Ser	Gly	Gly	Asp	Asn	Gly	Leu	Gly	Gly	His	Asn	Ala	Asn	Ser	
				20					25					30			
	Ala	Leu	Gly	Gln	Gln	Pro	Ile	Asp	Arg	Gln	Thr	Ile	Glu	Gln	Met	Ala	
			35					40					45				
	Gln	Leu	Leu	Ala	Glu	Leu	Leu	Lys	Ser	Leu	Leu	Ser	Pro	Gln	Ser	Gly	
		50					55					60					
20	Asn	Ala	Ala	Thr	Gly	Ala	Gly	Gly	Asn	Asp	Gln	Thr	Thr	Gly	Val	Gly	
	65				70						75				80		
	Asn	Ala	Gly	Gly	Leu	Asn	Gly	Arg	Lys	Gly	Thr	Ala	Gly	Thr	Thr	Pro	
					85					90				95			
25	Gln	Ser	Asp	Ser	Gln	Asn	Met	Leu	Ser	Glu	Met	Gly	Asn	Asn	Gly	Leu	
				100				105					110				
	Asp	Gln	Ala	Ile	Thr	Pro	Asp	Gly	Gln	Gly	Gly	Gly	Gln	Ile	Gly	Asp	
			115					120					125				
	Asn	Pro	Leu	Leu	Lys	Ala	Met	Leu	Lys	Leu	Ile	Ala	Arg	Met	Met	Asp	
		130					135					140					
30	Gly	Gln	Ser	Asp	Gln	Phe	Gly	Gln	Pro	Gly	Thr	Gly	Asn	Asn	Ser	Ala	
	145				150					155					160		
	Ser	Ser	Gly	Thr	Ser	Ser	Ser	Gly	Gly	Ser	Pro	Phe	Asn	Asp	Leu	Ser	
				165						170					175		
35	Gly	Gly	Lys	Ala	Pro	Ser	Gly	Asn	Ser	Pro	Ser	Gly	Asn	Tyr	Ser	Pro	
				180				185					190				
	Val	Ser	Thr	Phe	Ser	Pro	Pro	Ser	Thr	Pro	Thr	Ser	Pro	Thr	Ser	Pro	
			195					200					205				
	Leu	Asp	Phe	Pro	Ser	Ser	Pro	Thr	Lys	Ala	Ala	Gly	Gly	Ser	Thr	Pro	
		210					215					220					
40	Val	Thr	Asp	His	Pro	Asp	Pro	Val	Gly	Ser	Ala	Gly	Ile	Gly	Ala	Gly	
	225				230						235				240		
	Asn	Ser	Val	Ala	Phe	Thr	Ser	Ala	Gly	Ala	Asn	Gln	Thr	Val	Leu	His	
				245						250					255		

	Asp	Thr	Ile	Thr	Val	Lys	Ala	Gly	Gln	Val	Phe	Asp	Gly	Lys	Gly	Gln	
				260					265					270			
	Thr	Phe	Thr	Ala	Gly	Ser	Glu	Leu	Gly	Asp	Gly	Gly	Gln	Ser	Glu	Asn	
			275					280					285				
5	Gln	Lys	Pro	Leu	Phe	Ile	Leu	Glu	Asp	Gly	Ala	Ser	Leu	Lys	Asn	Val	
		290					295					300					
	Thr	Met	Gly	Asp	Asp	Gly	Ala	Asp	Gly	Ile	His	Leu	Tyr	Gly	Asp	Ala	
	305					310					315					320	
10	Lys	Ile	Asp	Asn	Leu	His	Val	Thr	Asn	Val	Gly	Glu	Asp	Ala	Ile	Thr	
				325						330					335		
	Val	Lys	Pro	Asn	Ser	Ala	Gly	Lys	Lys	Ser	His	Val	Glu	Ile	Thr	Asn	
			340						345					350			
	Ser	Ser	Phe	Glu	His	Ala	Ser	Asp	Lys	Ile	Leu	Gln	Leu	Asn	Ala	Asp	
			355					360					365				
15	Thr	Asn	Leu	Ser	Val	Asp	Asn	Val	Lys	Ala	Lys	Asp	Phe	Gly	Thr	Phe	
		370					375					380					
	Val	Arg	Thr	Asn	Gly	Gly	Gln	Gln	Gly	Asn	Trp	Asp	Leu	Asn	Leu	Ser	
	385					390					395					400	
20	His	Ile	Ser	Ala	Glu	Asp	Gly	Lys	Phe	Ser	Phe	Val	Lys	Ser	Asp	Ser	
				405						410					415		
	Glu	Gly	Leu	Asn	Val	Asn	Thr	Ser	Asp	Ile	Ser	Leu	Gly	Asp	Val	Glu	
			420						425					430			
25	Asn	His	Tyr	Lys	Val	Pro	Met	Ser	Ala	Asn	Leu	Lys	Val	Ala	Glu		
			435					440					445				

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa. The DNA molecule encoding this hypersensitive response elicitor protein or polypeptide has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

	atgtcaattc	ttacgcttaa	caacaatacc	tcgtcctcgc	cgggtctgtt	ccagtcgggg	60
	ggggacaacg	ggcttggtgg	tcataatgca	aattctgcgt	tggggcaaca	acccatcgat	120
35	cggcaaacca	ttgagcaaat	ggctcaatta	ttggcggaac	tgtaaagtc	actgctatcg	180
	ccacaatcag	gtaatgcggc	aaccggagcc	ggtggcaatg	accagactac	aggagttggt	240
	aacgctggcg	gcctgaacgg	acgaaaaggc	acagcaggaa	ccactccgca	gtctgacagt	300
	cagaacatgc	tgagtgagat	gggcaacaac	gggctggatc	aggccatcac	gcccgatggc	360
	cagggcgggc	ggcagatcgg	cgataatcct	ttactgaaag	ccatgctgaa	gcttattgca	420
40	cgcatgatgg	acggccaaag	cgatcagttt	ggccaacctg	gtacggggcaa	caacagtgcc	480
	tcttcgggta	cttcttcatc	tggcggttcc	ccttttaacg	atctatcagg	ggggaaggcc	540
	ccttcgggca	actccccttc	cggcaactac	tctcccgctc	gtacctttctc	acccccatcc	600
	acgccaacgt	cccctacctc	accgcttgat	ttcccttctt	ctcccaccaa	agcagccggg	660

ggcagcacgc cggtaacgga tcatacctgac cctgttggtgta gcgcggggcat cggggccgga 720  
 aattcgggtgg ccttcaccag cgccggcgct aatcagacgg tgctgcatga caccattacc 780  
 gtgaaagcgg gtcaggtggt tgatggcaaa ggacaaacct tcaccgccgg ttcagaatta 840  
 ggcgatggcg gccagtctga aaaccagaaa ccgctgttta tactggaaga cgggtgccagc 900  
 5 ctgaaaaacg tcaccatggg cgacgacggg gcggatggta ttcactctta cgggtgatgcc 960  
 aaaatagaca atctgcacgt caccaacgtg ggtgaggacg cgattaccgt taagccaaac 1020  
 agcgcgggca aaaaatccca cggtgaaatc actaacagtt ccttcgagca cgctctgac 1080  
 aagatcctgc agctgaatgc cgatactaac ctgagcggtg acaacgtgaa ggccaaagac 1140  
 tttggtactt ttgtacgcac taacggcggt caacagggta actgggatct gaatctgagc 1200  
 10 catatcagcg cagaagacgg taagtctctg ttcgttaaaa gcgatagcga ggggctaaac 1260  
 gtcaatacca gtgatatctc actgggtgat gttgaaaacc actacaaagt gccgatgtcc 1320  
 gccaacctga aggtggctga atga 1344

The above nucleotide and amino acid sequences are disclosed and further described in  
 15 U.S. Patent Application Serial No. 09/120,927 to Beer et al., which is hereby  
 incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from  
*Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 7  
 as follows:

20 Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met  
 1 5 10 15  
 Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser  
 20 25 30  
 25 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met  
 35 40 45  
 Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala  
 50 55 60  
 30 Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val  
 65 70 75 80  
 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe  
 85 90 95  
 Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met  
 100 105 110  
 35 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu  
 115 120 125  
 Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met  
 130 135 140  
 40 Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro  
 145 150 155 160  
 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe  
 165 170 175

	Leu	Asp	Gly	Asp	Glu	Thr	Ala	Ala	Phe	Arg	Ser	Ala	Leu	Asp	Ile	Ile
			180						185					190		
	Gly	Gln	Gln	Leu	Gly	Asn	Gln	Gln	Ser	Asp	Ala	Gly	Ser	Leu	Ala	Gly
			195					200					205			
5	Thr	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ser	Phe	Ser	Asn	Asn	Ser	Ser
		210					215					220				
	Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser
	225					230					235				240	
10	Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp
					245					250					255	
	Arg	Gly	Leu	Gln	Ser	Val	Leu	Ala	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Val
				260					265					270		
	Asn	Thr	Pro	Gln	Thr	Gly	Thr	Ser	Ala	Asn	Gly	Gly	Gln	Ser	Ala	Gln
			275					280					285			
15	Asp	Leu	Asp	Gln	Leu	Leu	Gly	Gly	Leu	Leu	Leu	Lys	Gly	Leu	Glu	Ala
	290						295					300				
	Thr	Leu	Lys	Asp	Ala	Gly	Gln	Thr	Gly	Thr	Asp	Val	Gln	Ser	Ser	Ala
	305					310					315					320
20	Ala	Gln	Ile	Ala	Thr	Leu	Leu	Val	Ser	Thr	Leu	Leu	Gln	Gly	Thr	Arg
					325					330					335	
	Asn	Gln	Ala	Ala	Ala											
					340											

25 This hypersensitive response elicitor protein or polypeptide has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., et al., "*Pseudomonas syringae* pv. *syringae* Harpin<sub>PS</sub>: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby

30 incorporated by reference in its entirety. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 8 as follows:

	atgcagagtc	tcagtcttaa	cagcagctcg	ctgcaaacc	cggcaatggc	ccttgctctg	60
35	gtacgtcctg	aagccgagac	gactggcagt	acgtcgagca	aggcgcttca	ggaagttgtc	120
	gtgaagctgg	ccgaggaact	gatgcgcaat	ggtcaactcg	acgacagctc	gccattggga	180
	aaactgttgg	ccaagtcgat	ggccgcagat	ggcaaggcgg	gcggcggtat	tgaggatgtc	240
	atcgctgcgc	tggacaagct	gatccatgaa	aagctcggtg	acaacttcgg	cgcgtctgcg	300
	gacagcgcc	cgggtaccgg	acagcaggac	ctgatgactc	aggtgctcaa	tggcctggcc	360
40	aagtcgatgc	tcgatgatct	tctgaccaag	caggatggcg	ggacaagctt	ctccgaagac	420
	gatatgccga	tgctgaacaa	gatcgcgag	ttcatggatg	acaatcccgc	acagtttccc	480



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aagccggact cgggctcctg ggtgaacgaa ctcaaggaag acaacttcct tgatggcgac 540
gaaacggctg cgttccgttc ggcactcgac atcattggcc agcaactggg taatcagcag 600
agtgacgctg gcagtctggc agggacgggt ggaggtctgg gcaactccgag cagtttttcc 660
aacaactcgt ccgtgatggg tgatccgctg atcgacgcca ataccggtcc cggtgacagc 720
5 ggcaataccc gtggtgaagc ggggcaactg atcggcgagc ttatcgaccg tggcctgcaa 780
tcggtattgg ccggtggtgg actgggcaca cccgtaaaca ccccgagac cgttacgtcg 840
gcgaatggcg gacagtccgc tcaggatctt gatcagttgc tgggcggctt gctgctcaag 900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960
gcgcaaatcg ccaccttgct ggtcagtacg ctgctgcaag gcacccgcaa tcaggctgca 1020
10 gcctga 1026

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The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,708,139 to Collmer et al. and U.S. Patent No. 5,776,889 to Wei et al., which are hereby incorporated by reference in their entirety.

15 Another hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

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20 Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
   1           5           10           15
   Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
           20           25           30
   Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
           35           40           45
25 Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
   50           55           60
   Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
   65           70           75           80
30 Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
           85           90           95
   Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
           100          105          110
   Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
           115          120          125
35 Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
   130           135          140
   Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
   145          150          155          160
40 Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
           165          170          175
   Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
           180          185          190

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	Pro	Gln	Ile	Thr	Pro	Gln	Leu	Ala	Asn	Pro	Asn	Arg	Thr	Ser	Gly	Thr	
			195					200					205				
	Gly	Ser	Val	Ser	Asp	Thr	Ala	Gly	Ser	Thr	Glu	Gln	Ala	Gly	Lys	Ile	
		210					215					220					
5	Asn	Val	Val	Lys	Asp	Thr	Ile	Lys	Val	Gly	Ala	Gly	Glu	Val	Phe	Asp	
	225					230					235					240	
	Gly	His	Gly	Ala	Thr	Phe	Thr	Ala	Asp	Lys	Ser	Met	Gly	Asn	Gly	Asp	
					245					250					255		
10	Gln	Gly	Glu	Asn	Gln	Lys	Pro	Met	Phe	Glu	Leu	Ala	Glu	Gly	Ala	Thr	
				260					265					270			
	Leu	Lys	Asn	Val	Asn	Leu	Gly	Glu	Asn	Glu	Val	Asp	Gly	Ile	His	Val	
			275					280					285				
	Lys	Ala	Lys	Asn	Ala	Gln	Glu	Val	Thr	Ile	Asp	Asn	Val	His	Ala	Gln	
		290					295					300					
15	Asn	Val	Gly	Glu	Asp	Leu	Ile	Thr	Val	Lys	Gly	Glu	Gly	Gly	Ala	Ala	
	305					310					315					320	
	Val	Thr	Asn	Leu	Asn	Ile	Lys	Asn	Ser	Ser	Ala	Lys	Gly	Ala	Asp	Asp	
					325					330					335		
20	Lys	Val	Val	Gln	Leu	Asn	Ala	Asn	Thr	His	Leu	Lys	Ile	Asp	Asn	Phe	
				340					345					350			
	Lys	Ala	Asp	Asp	Phe	Gly	Thr	Met	Val	Arg	Thr	Asn	Gly	Gly	Lys	Gln	
			355					360					365				
	Phe	Asp	Asp	Met	Ser	Ile	Glu	Leu	Asn	Gly	Ile	Glu	Ala	Asn	His	Gly	
		370					375					380					
25	Lys	Phe	Ala	Leu	Val	Lys	Ser	Asp	Ser	Asp	Asp	Leu	Lys	Leu	Ala	Thr	
	385					390					395					400	
	Gly	Asn	Ile	Ala	Met	Thr	Asp	Val	Lys	His	Ala	Tyr	Asp	Lys	Thr	Gln	
					405					410						415	
30	Ala	Ser	Thr	Gln	His	Thr	Glu	Leu									

This protein or polypeptide is acidic, glycine-rich, lacks cysteine, and is deficient in aromatic amino acids. The DNA molecule encoding this hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ.

ID. No. 10 as follows:

	tccacttcgc	tgattttgaa	attggcagat	tcatagaaac	gttcaggtgt	ggaaatcagg	60
	ctgagtgcgc	agatttcggt	gataagggtg	tggtactggt	cattgttggt	catttcaagg	120
	cctctgagtg	cgggtcggag	caataccagt	cttcctgctg	gcgtgtgcac	actgagtcgc	180
40	aggcataggc	atttcagttc	cttgcggttg	ttgggcata	aaaaaaagga	acttttataaa	240
	acagtgcaat	gagatgccgg	caaaacggga	accggtcgct	gcgctttgcc	actcacttcg	300
	agcaagctca	accccaaaca	tccacatccc	tatcgaacgg	acagcgatac	ggccacttgc	360
	tctggtaaac	cctggagctg	gcgtcggtcc	aattgccac	ttagcgaggt	aacgcagcat	420
	gagcatcggc	atcacacccc	ggccgcaaca	gaccaccacg	ccactcgatt	tttcggcgct	480

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aagcggcaag agtcctcaac caaacacgtt cggcgagcag aacactcagc aagcgatcga 540
cccgagtgca ctgttggttcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga 600
cagcacccgtc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc 660
taaattgatc agtgcattga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa 720
5  gcaggacacc aatcaggaac agcctgatag ccaggctcct ttccagaaca acggcgggct 780
cggtacaccg tcggccgata gcggggggcg cggtacaccg gatgcgacag gtggcgccgg 840
cggtgatacg ccaagcgcaa caggcggtgg cggcggtgat actccgaccg caacaggcgg 900
tggcggcagc ggtggcggcg gcacaccac tgcaacaggt ggcggcagcg gtggcacacc 960
cactgcaaca ggcggtggcg aggggtggcg aacaccgcaa atcactccgc agttggccaa 1020
10 ccctaaccgt acctcagga ctggctcggg gtcggacacc gcaggttcta ccgagcaagc 1080
cggcaagatc aatgtggtga aagacaccat caaggctcggc gctggcgaag tctttgacgg 1140
ccacggcgca accttcaactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca 1200
gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260
cgaggtcgat ggcattccacg tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320
15 gcatgccag aacgtcgggtg aagacctgat tacggtcaaa ggcgaggag gcgcagcggg 1380
cactaatctg aacatcaaga acagcagtgc caaagggtga gacgacaagg ttgtccagct 1440
caacgccaac actcacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggg 1500
tcgcaccaac ggtggcaagc agtttgatga catgagcatc gagctgaacg gcatcgaagc 1560
taaccacggc aagttcgccc tggtgaaaag cgacagtgc gatctgaagc tggcaacggg 1620
20 caacatcgcc atgaccgacg tcaaacacgc ctacgataaa acccaggcat cgaccaaca 1680
caccgagctt tgaatccaga caagtagctt gaaaaaaggg ggtggactc 1729

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The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent Application Serial No. 09/120,817 to Collmer et al., which is hereby incorporated by reference in its entirety.

A hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

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30  Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
    1           5           10           15
    Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
        20           25           30
    Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
        35           40           45
    35  Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
        50           55           60
    Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
        65           70           75           80
    40  Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
        85           90           95

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	Ala	Asn	Lys	Thr	Gly	Asn	Val	Asp	Asp	Ala	Asn	Asn	Gln	Asp	Pro	Met	
				100					105					110			
	Gln	Ala	Leu	Met	Gln	Leu	Leu	Glu	Asp	Leu	Val	Lys	Leu	Leu	Lys	Ala	
			115					120					125				
5	Ala	Leu	His	Met	Gln	Gln	Pro	Gly	Gly	Asn	Asp	Lys	Gly	Asn	Gly	Val	
		130					135					140					
	Gly	Gly	Ala	Asn	Gly	Ala	Lys	Gly	Ala	Gly	Gly	Gln	Gly	Gly	Leu	Ala	
	145					150					155					160	
10	Glu	Ala	Leu	Gln	Glu	Ile	Glu	Gln	Ile	Leu	Ala	Gln	Leu	Gly	Gly	Gly	
				165						170					175		
	Gly	Ala	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Gly	Val	Gly	Gly	Ala	Gly	Gly	
			180						185					190			
	Ala	Asp	Gly	Gly	Ser	Gly	Ala	Gly	Gly	Ala	Gly	Gly	Ala	Asn	Gly	Ala	
			195					200					205				
15	Asp	Gly	Gly	Asn	Gly	Val	Asn	Gly	Asn	Gln	Ala	Asn	Gly	Pro	Gln	Asn	
	210						215					220					
	Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Asp	Gly	Ser	Glu	Asp	
	225					230				235						240	
20	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn	
				245						250					255		
	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Gly	Asn	Gln	
				260					265					270			
	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly	
			275					280					285				
25	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser	
		290					295					300					
	Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val	
	305					310					315					320	
30	Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Gly	Ser	Gln	
				325						330					335		
	Gln	Ser	Thr	Ser	Thr	Gln	Pro	Met									
				340													

Further information regarding this hypersensitive response elicitor protein or polypeptide derived from *Pseudomonas solanacearum* is set forth in Arlat, M., et al., "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference in its entirety. It is encoded by a DNA molecule from *Pseudomonas solanacearum* having a nucleotide sequence corresponding SEQ. ID. No. 12 as follows:

40	atgtcagtcg	gaaacatcca	gagcccgtcg	aacctccgg	gtctgcagaa	cctgaacctc	60
	aacaccaaca	ccaacagcca	gcaatcgggc	cagtccgtgc	aagacctgat	caagcaggtc	120
	gagaaggaca	tcctcaacat	catcgcagcc	ctcgtgcaga	aggccgcaca	gtcggcgggc	180

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ggcaacaccg gtaacaccgg caacgcgccg gcgaaggacg gcaatgccaa cgcgggcgcc 240
aacgacccga gcaagaacga cccgagcaag agccaggctc cgcagtcggc caacaagacc 300
ggcaacgtcg acgacgccaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa 360
gacctggtga agctgctgaa ggcggccctg cacatgcagc agcccggcgg caatgacaag 420
5 ggcaacggcg tgggcggtgc caacggcgcc aagggtgccg gcggccaggg cggcctggcc 480
gaagcgctgc aggagatcga gcagatcctc gcccagctcg gcggcgggcg tgctggcgcc 540
ggcgggcgcg gtggcggtgt cggcggtgct ggtggcgcg atggcggtc cggcgcggt 600
ggcgcgaggc gtgcgaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac 660
ggccccgaga acgcaggcga tgtcaacggg gccaacggcg cggatgacgg cagcgaagac 720
10 cagggcgggc tcaccggcgt gctgcaaaag ctgatgaaga tcctgaacgc gctggtgcag 780
atgatgcagc aaggcggcct cggcgggcggc aaccaggcgc agggcggtc gaagggtgcc 840
ggcaacgcct cgccggcttc cggcgcgaa cggggcgga accagcccg ttcggcggt 900
gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc 960
gtccagatcc tgcagcagat gctggcgggc cagaacggcg gcagccagca gtccacctcg 1020
15 acgcagccga tgtaa 1035

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The above nucleotide and amino acid sequences are disclosed and further described in U.S. Patent No. 5,776,889 to Wei et al., which is hereby incorporated by reference in its entirety.

Other embodiments of the present invention include, but are not limited to, use of hypersensitive response elicitor proteins or polypeptides derived from *Erwinia carotovora* and *Erwinia stewartii*. Isolation of an *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui, et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrpN<sub>Ecc</sub>* and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPML, 9(7):565-73 (1996), which is hereby incorporated by reference in its entirety. A hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference in their entirety.

Hypersensitive response elicitor proteins or polypeptides from various *Phytophthora* species are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993); Ricci, et

al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989); Ricci, et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992); Baillreul, et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defense Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet, et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference in their entirety.

Another hypersensitive response elicitor protein or polypeptide which can be used in accordance with the present invention is derived from *Clavibacter michiganensis* subsp. *sepedonicus* and is described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference in its entirety.

Fragments of the above hypersensitive response elicitor proteins or polypeptides as well as fragments of full length elicitors from other pathogens can also be used according to the present invention.

Suitable fragments can be produced by several means. Subclones of the gene encoding a known elicitor protein can be produced using conventional molecular genetic manipulation for subcloning gene fragments, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), and Ausubel et al. (ed.), Current Protocols in Molecular Biology, John Wiley & Sons (New York, NY) (1999 and preceding editions), which are hereby incorporated by reference in their entirety. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or polypeptide that can be tested for elicitor activity, e.g., using procedures set forth in Wei, Z-M., et al., Science 257: 85-88 (1992), which is hereby incorporated by reference in its entirety.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. Erlich, H.A., et al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference in

its entirety. These can then be cloned into an appropriate vector for expression of a truncated protein or polypeptide from bacterial cells as described above.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which elicit a hypersensitive response are fragments of the *Erwinia amylovora* hypersensitive response elicitor protein or polypeptide of SEQ. ID. No. 3. The fragments can be a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180. DNA molecules encoding these fragments can also be utilized in the chimeric gene of the present invention.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the

protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor proteins or polypeptides used in accordance with the present invention are preferably produced in purified form (preferably at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is secreted into the growth medium of recombinant host cells (discussed *infra*). Alternatively, the protein or polypeptide of the present invention is produced but not secreted into growth medium. In such cases, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the hypersensitive response elicitor protein or polypeptide of interest is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by HPLC.

Other hypersensitive response elicitors can be readily identified by isolating putative protein or polypeptide candidates and testing them for elicitor activity as described, for example, in Wei, Z-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference in its entirety. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e., local necrosis) by using them to infiltrate appropriate plant tissues. Once identified, DNA molecules encoding a hypersensitive response elicitor can be isolated using standard techniques known to those skilled in the art.

DNA molecules encoding other hypersensitive response elicitor proteins or polypeptides can also be identified by determining whether such DNA molecules hybridizes under stringent conditions to a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2, 4, 6, 8, 10, or 12. An example of suitable stringency conditions is when hybridization is carried out at a temperature of about 37°C using a hybridization medium that includes 0.9M sodium citrate ("SSC") buffer, followed by washing with 0.2x SSC buffer at 37°C. Higher stringency can readily be attained by increasing the temperature for either hybridization or washing conditions



or increasing the sodium concentration of the hybridization or wash medium.

Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Exemplary high stringency conditions include carrying out hybridization at a temperature of about 42°C to about 65°C for up to about 20 hours in a hybridization medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate (SDS), 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and 50 µg/ml *E. coli* DNA, followed by washing carried out at between about 42°C to about 65°C in a 0.2x SSC buffer.

~~The DNA molecule encoding the hypersensitive response elicitor~~ polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which

is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference in its entirety), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference in its entirety.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eukaryotic promoters differ from those of prokaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a prokaryotic system, and, further, prokaryotic promoters are not recognized and do not function in eukaryotic cells.

Similarly, translation of mRNA in prokaryotes depends upon the presence of the proper prokaryotic signals which differ from those of eukaryotes. Efficient translation of mRNA in prokaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG,

which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see  
5 Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference in its entirety.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression  
10 of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the  $P_R$  and  $P_L$  promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*,  
15 and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which  
20 inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene  
25 transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong"  
30 transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not

limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

5               Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast,  
10               mammalian cells, insect, plant, and the like.

              Because it is desirable for recombinant host cells to secrete the hypersensitive response elicitor protein or polypeptide, it is preferable that the host cell also be transformed with a type III secretion system in accordance with Ham et al., "A Cloned *Erwinia chrysanthemi* Hrp (Type III Protein Secretion) System  
15               Functions in *Escherichia coli* to Deliver *Pseudomonas syringae* Avr Signals to Plant Cells and Secrete Avr Proteins in Culture," Microbiol. 95:10206-10211 (1998), which is hereby incorporated by reference in its entirety.

              Isolation of the hypersensitive response elicitor protein or polypeptide from the host cell or growth medium can be carried out as described above.

20               The methods of the present invention can be performed by treating the fruit or vegetable either prior to or after harvest of the fruit or vegetable.

              Suitable preharvest application methods include, without limitation, high or low pressure spraying of the entire plant and fruits. Suitable postharvest application methods include, without limitation, low or high pressure spraying,  
25               coating, or immersion. Other suitable application procedures (both preharvest and postharvest) can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with the fruit or vegetable. Once treated, the fruits or vegetables can be handled, packed, shipped, and processed using conventional procedures to deliver the produce to  
30               processing plants or end-consumers.

              The hypersensitive response elicitor polypeptide or protein can be applied to fruits or vegetables in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

polypeptide or protein can be applied separately to fruits or vegetables with other materials being applied at different times.

5 A composition suitable for treating fruits or vegetables in accordance with the application embodiment of the present invention contains an isolated hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. The composition preferably contains greater than about 500 nM hypersensitive response elicitor polypeptide or protein, although greater or lesser amounts of the hypersensitive response elicitor polypeptide or protein depending on the rate of composition application and efficacy of different hypersensitive response elicitor proteins or polypeptides.

10 Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include  $(\text{NH}_4)_2\text{NO}_3$ . An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

15 Other suitable additives include buffering agents, wetting agents, coating agents, and ripening agents. These materials can be used either to facilitate the process of the present invention or to provide additive benefits to inhibit postharvest disease and desiccation.

20 As indicated above, one embodiment of the present invention involves treating fruits or vegetables with an isolated hypersensitive response elicitor protein or polypeptide. The hypersensitive response elicitor protein or polypeptide can be isolated from its natural source (e.g., *Erwinia amylovora*, *Pseudomonas syringae*, etc.) or from recombinant source transformed with a DNA molecule encoding the protein or polypeptide.

25 Another aspect of the present invention relates to a DNA construct as well as host cells, expression systems, and transgenic plants which contain the heterologous DNA construct.

30 The DNA construct includes a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, a plant-expressible promoter operably coupled 5' to the DNA molecule and which is effective to transcribe the DNA molecule in fruit or vegetable tissue, and a 3' regulatory region operably coupled to the DNA molecule. Expression of the DNA molecule in fruit or vegetable

tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation.

Expression of such heterologous DNA molecules requires a suitable promoter which is operable in plant tissues. In some embodiments of the present invention, it may be desirable for the heterologous DNA molecule to be expressed in many, if not all, tissues. Such promoters yield constitutive expression of coding sequences under their regulatory control. Exemplary constitutive promoters include, without limitation, the nopaline synthase promoter (Fraley et al., Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 35S promoter (O'Dell et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

While constitutive expression is generally suitable for expression of the DNA molecule, it should be apparent to those of skill in the art that temporally or tissue regulated expression may also be desirable, in which case any regulated promoter can be selected to achieve the desired expression. Typically, the temporally or tissue regulated promoters will be used in connection with the DNA molecule that are expressed at only certain stages of development or only in certain tissues.

In another embodiment of the present invention, expression of the heterologous DNA molecule is directed in a tissue-specific manner or environmentally-regulated manner (i.e., inducible promoters). Tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues.

For example, the E4 and E8 promoters of tomato have been used to direct fruit-specific expression of a heterologous DNA sequence in transgenic tomato plants (Cordes et al., Plant Cell 1:1025-1034 (1989); Deikman et al., EMBO J. 7:3315-3320 (1988); and Della Penna et al., Proc. Natl. Acad. Sci. USA 83:6420-6424 (1986), which are hereby incorporated by reference in their entirety). Another fruit-specific promoter is the PG promoter (Bird et al., Plant Molec. Biol. 11:651-662 (1988), which is hereby incorporated by reference in its entirety). Another tissue-specific promoter is the AP2 promoter from the ovule-specific BEL1 gene promoter

described in Reiser et al., Cell 83:735-742 (1995), which is hereby incorporated by reference in its entirety.

Promoters useful for expression in seed tissues include, without limitation, the promoters from genes encoding seed storage proteins, such as napin, cruciferin, phaseolin, and the like (see U.S. Patent No. 5,420,034 to Kridl et al., which is hereby incorporated by reference in its entirety). Other suitable promoters include those from genes encoding embryonic storage proteins.

Promoters useful for expression in leaf tissue include the Rubisco small subunit promoter.

Promoters useful for expression in tubers, particularly potato tubers, include the patatin promoter.

~~Examples of environmental conditions that may affect transcription by~~ inducible promoters include anaerobic conditions, elevated temperature, or the presence of light. In some plants, it may also be desirable to use promoters which are responsive to pathogen infiltration or stress. For example, it may be desirable to limit expression of the protein or polypeptide in response to infection by a particular pathogen of the plant. One example of a pathogen-inducible promoter is the *gstI* promoter from potato, which is described in U.S. Patent Nos. 5,750,874 and 5,723,760 to Strittmayer et al., which are hereby incorporated by reference in their entirety.

Expression of the DNA molecule in isolated plant cells or tissue or whole plants also utilizes appropriate transcription termination and polyadenylation of mRNA. Any 3' regulatory region suitable for use in plant cells or tissue can be operably linked to the first and second DNA molecules. A number of 3' regulatory regions are known to be operable in plants. Exemplary 3' regulatory regions include, without limitation, the nopaline synthase 3' regulatory region (Fraley, et al., "Expression of Bacterial Genes in Plant Cells," Proc. Nat'l. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety) and the cauliflower mosaic virus 3' regulatory region (Odell, et al., "Identification of DNA Sequences Required for Activity of the Cauliflower Mosaic Virus 35S Promoter," Nature, 313(6005):810-812 (1985), which is hereby incorporated by reference in its entirety).

5 The promoter and a 3' regulatory region can readily be ligated to the DNA molecule using well known molecular cloning techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), which is hereby incorporated by reference in its entirety.

10 One approach to transforming plant cells with a DNA molecule of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford, et al., which are hereby incorporated by reference in their entirety. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

20 Another method of introducing the DNA molecule into plant cells is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the DNA molecule. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference in its entirety.

25 The DNA molecule may also be introduced into the plant cells by electroporation. Fromm, et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference in its entirety. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the DNA molecule.

30 Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.



Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the DNA molecule. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

*Agrobacterium* is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences such as a DNA molecule a hypersensitive response elicitor protein or polypeptide can be introduced into appropriate plant cells by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. Schell, J., Science, 237:1176-83 (1987), which is hereby incorporated by reference in its entirety.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers.

After transformation, the transformed plant cells can be selected and regenerated.

Preferably, transformed cells are first identified using, e.g., a selection marker simultaneously introduced into the host cells along with the DNA molecule of the present invention. Suitable selection markers include, without limitation, markers coding for antibiotic resistance, such as kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA, 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety). A number of antibiotic-resistance markers are known in the art and other are continually being identified. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection media containing an

antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow.

Once a recombinant plant cell or tissue has been obtained, it is possible to regenerate a full-grown plant therefrom. Thus, another aspect of the present invention relates to a transgenic plant that includes a heterologous DNA molecule encoding a hypersensitive response elicitor protein or polypeptide, wherein the heterologous DNA molecule is under control of a promoter that induces transcription of the DNA molecule fruit or vegetable tissues. Preferably, the DNA molecule is stably inserted into the genome of the transgenic plant of the present invention.

Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference in their entirety.

It is known that practically all plants can be regenerated from cultured cells or tissues, including both monocots and dicots.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the DNA molecule encoding the hypersensitive response elicitor protein or polypeptide is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars. With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the field.

With regard to the use of the hypersensitive response elicitor protein or polypeptide in imparting postharvest disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease can be reduced and symptom development can be delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of controlling postharvest disease has the potential for controlling previously untreatable diseases and avoiding the use of infectious agents or environmentally harmful materials.

With respect to desiccation, complete protection against desiccation may not be conferred, but the severity of desiccation can be reduced. Desiccation protection inevitably will depend, at least to some extent, on other conditions such as storage temperatures, light exposure, etc. However, this method of controlling desiccation has the potential for eliminating some other treatments (i.e., use of coating waxes) which may contribute to reduced costs or, at least, substantially no increase in costs.

The methods of the present invention can be used to control a number of postharvest diseases caused by a variety of pathogens. These postharvest diseases and the causative agents which can be treated according to the present invention include, without limitation, the following: *Penicillium* (e.g., *Penicillium digitatum*), *Botrytis* (e.g., *Botrytis cinerea*), *Phytophthora* (e.g., *Phytophthora citrophthora*), and *Erwinia* (e.g. *Erwinia carotovora*).

A further aspect of the present invention relates to a method of enhancing the longevity of fruit or vegetable ripeness.

According to one embodiment, this aspect of the present invention is carried out by treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhance the longevity of fruit or vegetable ripeness. Preferably, as noted above, the hypersensitive response elicitor protein or polypeptide is in isolated form. Treating of the fruit or vegetable can be performed either prior to harvest after harvest of the fruit or vegetable, using the techniques described above.

According to another embodiment, this aspect of the present invention is carried out by providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and then growing the transgenic plant or transgenic plant produced from the transgenic plant

seed under conditions effective to enhance the longevity of fruit or vegetable ripeness in a fruit or vegetable harvested from the transgenic plant. This aspect of the present invention may further include applying the hypersensitive response elicitor polypeptide or protein to the fruit or vegetable to enhance the longevity of fruit or vegetable ripeness. Treating of the fruit or vegetable can be performed either prior to harvest or after harvest of the fruit or vegetable, using the techniques described above.

The methods of the present invention can be utilized to treat a wide variety of fruits and vegetables to control postharvest disease or desiccation as well as enhance the longevity of fruit or vegetable ripeness. Fruits and vegetables which can be treated include any edible plant product, particularly those from traditional crop plants, such as seed, root, tuber, stem, leaf, flower, and fruit. Exemplary transgenic fruit plants and fruits that can be treated include, without limitation, apple, pear, peach, nectarine, apricot, plum, cherry, olive, melon, citrus, grape, strawberry, raspberry, blueberry, currant, pineapple, papaya, guava, banana, and kiwi. Exemplary transgenic vegetable plants and vegetables that can be treated include, without limitation, asparagus, potato, sweet potato, bean, pea, chicory, lettuce, parsley, basil, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, spinach, onion, garlic, eggplant, pepper, celery, leek, radish, carrot, squash, pumpkin, zucchini, cucumber, soybean, tobacco, tomato, sorghum, rhubarb, and sugarcane. Exemplary transgenic grain plants and grain products which can be treated include, without limitation, alfalfa, rice, wheat, barley, corn, and rye.

### EXAMPLES

The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope.

As used in the following Examples, Messenger<sup>®</sup> refers to a product available from Eden Bioscience Corporation (Bothell, Washington), which contains 3% by weight of harpin<sub>Ea</sub> as the active ingredient and 97% by weight inert ingredients. Harpin<sub>Ea</sub> is one type of hypersensitive response elicitor protein from *Erwinia amylovora*, identified herein by SEQ. ID. No. 3.

**Example 1 – Effect of Treating Orange Fruits with Messenger® on Postharvest Orange Storage**

On day 0, Fall-GLO orange fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on oranges were then dried by air, and the treated oranges were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated oranges was then put into a 18°C growth chamber for storage. On day 7, orange fruits were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of orange fruit. The above procedure was performed on 40 orange fruits per treatment.

Measurements of disease were conducted on days 20, 24, and 26 following treatment with Messenger® or buffer solution. Grades 0-5 indicate different disease scales -- Grade 0: No symptoms; Grade 1: 1/5 an individual fruit has disease symptoms; Grade 2: 2/5 an individual fruit has disease symptoms; Grade 3: 3/5 an individual fruit has disease symptoms; Grade 4: 4/5 an individual fruit has disease symptoms; Grade 5: whole fruit has disease symptoms. The results of these treatments are set forth in Table 1 below.

Table 1: Reduction of Disease Index in Oranges

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	20	33	3	1	0	2	1	0.09	58.14%	yes	yes
Buffer	20	23	8	0	2	6	1	0.22	n/a	-	-
Messenger®	24	25	2	6	4	1	2	0.20	45.21%	yes	yes
Buffer	24	16	7	3	3	4	7	0.37	n/a	-	-
Messenger®	26	19	4	6	4	5	2	0.29	36.96%	yes	yes
Buffer	26	16	3	3	0	7	11	0.46	n/a	-	-

The data listed in Table 1 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger® treatment can reduce orange disease about 58.14% at 21 days, about 45.21% at 25 days, and 36.97% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95%

and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 2 – Effect of Treating Tomato (Hot House) Fruits with Messenger<sup>®</sup> on Postharvest Tomato Storage**

On day 0, Hot House tomato fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger<sup>®</sup> or buffer solutions on tomatoes were then dried by air, and the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of tomato fruit. The above procedure was performed on 15 tomatoes fruits per treatment.

Measurements of disease were conducted on days 21 and 27 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 2 below.

Table 2: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger <sup>®</sup>	21	7	2	2	3	1	0	0.25	58.70%	yes	yes
Buffer	21	3	1	2	1	2	6	0.61	n/a	-	-
Messenger <sup>®</sup>	27	2	2	4	3	2	2	0.49	30.19%	yes	yes
Buffer	27	1	1	2	2	3	6	0.71	n/a	-	-

The data listed in Table 2 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger<sup>®</sup> treatment can reduce tomato disease about 58.70% at 21 days and about 30.19% at 27 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 3 – Effect of Treating Grape Fruits with Messenger® on Postharvest Grape Storage**

On day 0, Red G. Grape fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapes were then dried by air, and the treated grapes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapes was then put into a 18°C growth chamber for storage. On day 7, grapes were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of grape fruit. The above procedure was performed on about 3700g of grape fruits per treatment.

Measurements of disease were conducted on days 14 and 21 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 3 below.

Table 3: Reduction of Disease Index in Grapes

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	14	225	99	42	39	21	13	0.20	45.65%	yes	yes
Buffer	14	98	130	91	52	38	48	0.38	n/a	-	-
Messenger®	21	66	83	126	98	39	27	0.42	39.35%	yes	yes
Buffer	21	18	36	64	72	119	137	0.69	n/a	-	-

The data listed in Table 3 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger® treatment can reduce grape disease by about 45.65% at 14 days and about 39.35% at 21 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment® and buffer treatment.

**Example 4 – Effect of Treating Grapefruit Fruits with Messenger® on Postharvest Grapefruit Storage**

On day 0, FL 33935 grapefruit fruits were treated by spraying Messenger® solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger® or buffer solutions on grapefruits were then dried by air, and the treated grapefruits were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated grapefruit fruits was then put into a 18°C growth chamber for storage. On day 7, grapefruit fruits were inoculated with *Phytophthora citrophthora* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of grapefruit fruit. The above procedure was performed on 6 grapefruit fruits per treatment.

Measurements of disease were conducted on days 87, 97, 103, and 111 following treatment with Messenger® or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 4 below.

Table 4: Reduction of Disease Index in Grapefruits

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	87	5	1	0	0	0	0	0.03	75.00%	yes	yes
Buffer	87	4	1	0	1	0	0	0.13	n/a	-	-
Messenger®	97	5	0	0	1	0	0	0.10	50.00%	yes	yes
Buffer	97	4	0	1	0	1	0	0.20	n/a	-	-
Messenger®	103	4	1	0	0	1	0	0.17	28.57%	yes	yes
Buffer	103	3	2	0	0	0	1	0.23	n/a	-	-
Messenger®	111	4	1	0	0	0	1	0.20	33.33%	yes	yes
Buffer	111	3	1	0	1	0	1	0.30	n/a	-	-

The data listed in Table 4 above shows that the Messenger® was more effective than buffer as a fruit spray treatment in reducing disease index for *Phytophthora citrophthora* and providing longer storage life. Messenger® treatment can reduce grapefruit disease by about 75.00% at 87 days, about 50.00% at 97 days, about 28.57% at 103 days, and about 33.33% at 111 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant



differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 5 - Effect of Treating Apple (Fuji) Fruits with Messenger<sup>®</sup> on Postharvest Apple Storage**

On day 0, Fuji apple fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger<sup>®</sup> or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 5 below.

Table 5: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger <sup>®</sup>	50	20	0	0	0	0	0	0.00	100.00%	yes	yes
Buffer	50	18	1	1	0	0	0	0.03	n/a	-	-
Messenger <sup>®</sup>	61	19	1	0	0	0	0	0.01	88.89%	yes	yes
Buffer	61	16	2	1	0	0	1	0.09	n/a	-	-
Messenger <sup>®</sup>	70	18	0	2	0	0	0	0.04	71.43%	yes	yes
Buffer	70	14	2	2	1	0	1	0.14	n/a	-	-
Messenger <sup>®</sup>	78	15	2	3	0	0	0	0.08	57.89%	yes	yes
Buffer	78	13	2	2	1	0	2	0.19	n/a	-	-
Messenger <sup>®</sup>	85	13	3	1	1	2	0	0.16	40.74%	yes	yes
Buffer	85	10	5	0	0	3	2	0.27	n/a	-	-

The data listed in Table 5 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life.

Messenger<sup>®</sup> treatment can reduce apple disease by about 100.00% at 51 days, 88.89% at 61 days, 71.43% at 70 days, 57.89% at 78 days, and 40.74% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

**Example 6 - Effect of Treating Apple (Granny Smith) Fruits with Messenger<sup>®</sup> on Postharvest Apple Storage**

On day 0, Granny Smith apple fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. The Messenger<sup>®</sup> or buffer solutions on apples were then dried by air, and the treated apples were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated apples was then put into a 18°C growth chamber for storage. On day 7, apples were inoculated with *Penicillium digitatum* and *Phytophthora citrophora* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of apples. The above procedure was performed on 20 apples per treatment.

Measurements of disease were conducted on days 50, 61, 70, 78, and 85 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 6 below.

Table 6: Reduction of Disease Index in Apples

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger®	50	20	0	0	0	0	0	00.00	100.00%	yes	yes
Buffer	50	19	1	0	0	0	0	0.01	n/a	-	-
Messenger®	61	13	5	2	0	0	0	0.09	50.00%	yes	yes
Buffer	61	7	9	3	1	0	0	0.18	n/a	-	-
Messenger®	70	7	10	3	0	0	0	0.16	36.00%	yes	yes
Buffer	70	2	12	5	1	0	0	0.25	n/a	-	-
Messenger®	78	6	10	3	1	0	0	0.19	32.14%	yes	yes
Buffer	78	2	11	5	1	1	0	0.28	n/a	-	-
Messenger®	85	7	9	2	1	1	0	0.20	23.08	yes	yes
Buffer	85	4	10	4	1	0	1		n/a	-	-

The data listed in Table 6 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Phytophthora citrophora* and providing longer storage life. Messenger<sup>®</sup> treatment can reduce apple disease by about 100.00% at 51 days, 50.00% at 61 days, 36.00% at 70 days, 32.14% at 78 days, and 23.08% at 85 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger treatment<sup>®</sup> and buffer treatment.

#### 10 **Example 7 - Effect of Treating Tomato Fruits with Messenger<sup>®</sup> on Postharvest Tomato Storage**

On day 0, tomato fruits were treated by spraying Messenger<sup>®</sup> solution (ca. 15 ug/ml) or buffer solution (5mM KPO<sub>4</sub>, pH 6.8) on the surface of fruits in a 22°C greenhouse. After the Messenger<sup>®</sup> or buffer solutions on tomatoes were dried by air, the treated tomatoes were marked, mixed together, and put into a plastic container (Clear View 66 Qt/63L made by Sterilite Corporation). The container with treated tomatoes was then put into a 18°C growth chamber for storage. On day 7, tomatoes were inoculated with *Penicillium digitatum* and *Botrytis cinerea* by spraying a 10<sup>5</sup> cfu/ml suspension on the surface of tomatoes. The above procedure was performed on 44 tomatoes per treatment.

Measurements of disease were conducted on days 18, 27, 35, and 42 following treatment with Messenger<sup>®</sup> or buffer solution. Grades are indicated according to the criteria set forth in Example 1. The results of these treatments are set forth in Table 7 below.

Table 7: Reduction of Disease Index in Tomatoes

Sample	Days After Treatment	Grade						Index	Efficacy	T-test	
		0	1	2	3	4	5			p<0.05	p<0.01
Messenger <sup>®</sup>	18	21	18	5	0	0	0	0.13	37.78%	yes	yes
Buffer	18	11	21	12	0	0	0	0.20	n/a	-	-
Messenger <sup>®</sup>	27	16	18	9	1	0	0	0.18	25.00%	yes	yes
Buffer	27	8	24	8	4	0	0	0.24	n/a	-	-
Messenger <sup>®</sup>	35	7	14	13	10	0	0	0.32	16.67%	yes	yes
Buffer	35	1	16	15	10	2	0	0.38	n/a	-	-
Messenger <sup>®</sup>	42	1	10	9	12	9	3	0.52	12.88%	yes	yes
Buffer	42	0	3	15	10	11	5	0.60	n/a	-	-

The data listed in Table 7 above shows that the Messenger<sup>®</sup> was more effective than buffer as a fruit spray treatment in reducing disease index for *Penicillium digitatum* and *Botrytis cinerea* and providing longer storage life. Messenger<sup>®</sup> treatment can reduce tomato disease by about 37.78% at 18 days, 25.00% at 27 days, 16.67% at 35 days, and 12.88% at 42 days after spraying treatment and 18°C storage conditions. T-test shows that there are statistically significant differences at both 95% and 99% confidence levels for the results obtained from Messenger<sup>®</sup> treatment and buffer treatment.

**Example 8 - Effect of Preharvest and Postharvest Messenger<sup>®</sup> Treatments on Tomato (Sanibel) Fruit Postharvest Storage**

Plots of red and green Sanibel variety tomatoes were grown under either standard conditions or full Messenger<sup>®</sup> treatment over the course of the growing season. The standard conditions, also known as grower's standard, included fungicide treatment sprayed every seven days after transplanting using primarily fungicides containing copper-based active ingredients. The Messenger<sup>®</sup> treatment included six sprays at rate of 2.2 oz of the product per acre.

Red and green fruits were harvested from both the Messenger<sup>®</sup> treated and grower standard plots. It was noted that green tomatoes from the grower standard treatment plots were smaller (i.e. less mature) than green tomatoes from the messenger treated plants.

Harvested fruits were treated as follows:

- (1) Fruits from Messenger<sup>®</sup> treated plots were further treated with Messenger<sup>®</sup> after harvest;
- (2) Fruits from standard plots were treated with Messenger<sup>®</sup> after harvest;
- (3) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest; and
- (4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (1) and (2) was carried out by spraying with Messenger<sup>®</sup> at a rate of 20 ppm harpin<sub>Ea</sub> concentration using a

backpack-sprayer at about 30 p.s.i. The fruit were rolled during application to assure full coverage of the spray. The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in storage in a single layer. Storage temperatures ranged from about 18 to 32°C and light intervals were approximately 12 hours of light and darkness. Tomatoes were checked daily for rot and desiccation for a total of 31 days after harvest. The results are shown in Table 8 below.

Table 8: Affect of Preharvest and Postharvest Treatment on Rot and Desiccation

Group	Ripe- ness	No. Fruit	Days After Harvest							No. Desiccated	% Marketable
			14	19	21	22	23	25	31		
(1) Pre/Postharvest Messenger®	Red	5	0	0	0	1	1	1	2	0	60%
	Green	4	0	0	0	0	0	0	0	0	100%
(2) Postharvest Messenger® Only	Red	5	0	0	0	0	0	0	0	4	20%
	Green	4	0	0	0	0	0	0	0	1	75%
(3) Preharvest Messenger® Only	Red	5	0	0	0	0	0	0	2	0	60%
	Green	5	0	0	0	0	0	0	0	0	100%
(4) No Messenger®	Red	5	1	3	1	5	5	5	5	0	0%
	Green	5	0	0	0	0	0	0	0	1	80%

The red tomatoes from group (4) all rotted by day 21. In contrast, all red tomatoes which received some form of Messenger® treatment showed reduced rate of decay and rot. Near the end of the trial a number of tomatoes were observed to have desiccated, exhibiting shriveled skins but no rot. These were included as non-marketable. These results are suggestive that both preharvest and postharvest Messenger® treatments can reduce the level of rotting and desiccation, thereby extending fresh storage time.

#### **Example 9 - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato During Ambient Storage**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger® treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Three different treatment regimen were examined as follows:

- 5 (1) Fruits from Messenger® treated plots received no additional treatment following harvest;
- (2) Fruits from standard plots were treated with Messenger® after harvest;
- (3) Fruits from standard plots received no additional treatment following harvest.

10 Postharvest treatment of fruits from group (2) was carried out by dipping the fruit in a Messenger® solution (20 ppm harpin<sub>EA</sub>). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(3) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the rating scale below.

	<u>Grade</u>	<u>Description</u>
15	1	Mature Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;
	2	Pink: Initial sign of color break noticed on some areas of fruit; these areas are usually pink;
	3	Pink/Red: Intermediate ripening: Fruit is not total red; some pink still remains;
20	4	Red: Fruit totally red in color;
	5	Decay: Some areas of the fruit beginning to break down from decay.

The results of this test are summarized in Table 9 below.

Table 9: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade					Index	Efficacy	T-test	
		1	2	3	4	5			p<0.05	p<0.01
1	10	11	6	8	75	0	0.69	7.28%	yes	yes
2	10	5	7	11	77	0	0.72	3.81%	yes	yes
3	10	5	3	6	86	1	0.75	N/A	N/A	N/A
1	14	4	5	5	86	0	0.75	2.61%	yes	yes
2	14	2	6	5	87	0	0.75	1.57%	yes	yes
3	14	2	4	4	89	1	0.77	N/A	N/A	N/A
1	17	0	0	3	92	5	0.80	3.37%	yes	yes
2	17	0	1	4	82	13	0.81	2.16%	yes	yes
3	17	0	0	1	82	17	0.83	N/A	N/A	N/A
1	20	0	0	0	89	11	0.82	2.61%	yes	yes
2	20	0	0	0	80	20	0.84	0.47%	yes	yes
3	20	0	0	1	76	23	0.84	N/A	N/A	N/A

The data generated in this trial indicate that treatment of tomatoes with Messenger<sup>®</sup>, either through field sprays or as a post harvest dip, resulted in earlier fruit red ripening compared to grower's standard. In addition, although early ripening was observed, the Messenger<sup>®</sup> treatments maintained the red ripe condition longer than the grower's standard with delay of breakdown and decay.

#### **Example 10 - Effect on Messenger on Post Harvested Maturity and Fruit Decay of Tomato Under Cold Storage Conditions**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger<sup>®</sup> treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

- (1) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest;
- (2) Fruits from Messenger<sup>®</sup> treated plots were further treated with Messenger<sup>®</sup> after harvest;

- (3) Fruits from standard plots were treated with Messenger® after harvest; and
- (4) Fruits from standard plots received no additional treatment following harvest.

5

Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger® solution (20 ppm harpin<sub>EA</sub>). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage in a Custom Packing House cooler at 11°C (52°F). The tomatoes were then rated for color development and decay over time using the rating scale described in Example 8. The results of this study appear in Table 10 below.

10

Table 10: Affect of Preharvest and Postharvest Treatment on Maturity and Decay

Group	Days After Treatment	Grade					Index	Efficacy	T-test	
		1	2	3	4	5			p<0.05	p<0.01
1	7	66	34	0	0	0	0.27	0.00%	yes	yes
2	7	67	33	0	0	0	0.27	0.75%	yes	yes
3	7	76	24	0	0	0	0.27	7.46%	yes	yes
4	7	68	30	2	0	0	0.27	N/A	yes	yes
1	10	59	31	8	0	0	0.30	7.53%	yes	yes
2	10	60	28	12	0	0	0.30	5.00%	yes	yes
3	10	65	35	0	0	0	0.27	15.63%	yes	yes
4	10	49	42	9	0	0	0.32	N/A	N/A	N/A
1	17	19	35	28	18	0	0.49	7.20%	yes	yes
2	17	20	38	28	14	0	0.47	10.61%	yes	yes
3	17	19	28	39	14	0	0.50	6.06%	yes	yes
4	17	17	27	31	25	0	0.53	N/A	N/A	N/A
1	21	11	28	29	32	0	0.56	6.62%	N/A	N/A
2	21	15	26	37	22	0	0.53	11.92%	yes	yes
3	21	10	33	35	22	0	0.54	10.93%	yes	yes
4	21	10	18	32	40	0	0.60	N/A	N/A	N/A
1	26	3	15	23	59	0	0.68	-2.26%	yes	yes
2	26	9	19	25	41	6	0.63	4.39%	yes	yes
3	26	3	23	31	43	0	0.63	5.00%	yes	yes
4	26	2	19	23	50	1	0.66	N/A	N/A	N/A
1	32	3	15	23	59	0	0.68	-2.26%	yes	yes
2	32	9	19	25	41	6	0.63	4.39%	yes	yes
3	32	3	23	31	43	0	0.63	5.00%	yes	yes
4	32	2	19	23	50	1	0.66	N/A	N/A	N/A



Table 10 cont.

Group	Days After Treatment	Grade					Index	Efficacy	T-test	
		1	2	3	4	5			p<0.05	p<0.01
1	38	0	4	10	84	2	0.77	0.26%	yes	yes
2	38	1	10	15	65	9	0.74	3.64%	yes	yes
3	38	1	5	14	78	2	0.75	2.60%	yes	yes
4	38	0	3	13	80	4	0.77	N/A	N/A	N/A
1	45	0	3	11	74	12	0.79	2.95%	yes	yes
2	45	1	4	12	69	14	0.78	3.93%	yes	yes
3	45	0	1	11	81	7	0.79	3.19%	yes	yes
4	45	0	0	10	73	17	0.81	N/A	N/A	N/A
1	50	0	3	10	63	23	0.82	3.55%	yes	yes
2	50	0	4	11	58	27	0.82	3.55%	yes	yes
3	50	0	0	8	78	14	0.81	4.02%	yes	yes
4	50	0	0	3	71	26	0.85	N/A	N/A	N/A
1	55	0	0	0	73	27	0.85	1.84%	yes	yes
2	55	0	0	0	68	32	0.86	0.69%	yes	yes
3	55	0	0	2	80	18	0.83	4.37%	yes	yes
4	55	0	0	0	65	35	0.87	N/A	N/A	N/A
1	60	0	0	0	65	35	0.87	2.47%	yes	yes
2	60	0	0	0	63	37	0.87	2.02%	yes	yes
3	60	0	0	0	74	26	0.85	4.48%	yes	yes
4	60	0	0	0	54	46	0.89	N/A	N/A	N/A
1	65	0	0	0	53	47	0.89	1.76%	yes	yes
2	65	0	0	0	58	42	0.88	2.86%	yes	yes
3	65	0	0	0	65	35	0.87	4.40%	yes	yes
4	65	0	0	0	45	55	0.91	N/A	N/A	N/A

5 In previous trials when tomatoes were treated with Messenger® in the field and/or with a post harvest dip, the fruit appeared to develop to red ripe more quickly than the grower's standard, when held at ambient temperatures (75-80°F). Although this early ripening was observed, these red fruit did not begin to decay earlier than the grower's standard. In this study, the fruit were held at a constant 52°F in a commercial cold storage room at a tomato packinghouse facility. It appears that

10 this lower temperature slows the ripening process, as would be expected, and Messenger® treatments did not increase the rate of the red ripening for the first 30 days, as observed in previous tests. The Messenger® treatments did, however,

seem to maintain the red ripe condition longer than the grower's standard without breakdown and decay.

**Example 11 - Effect of Messenger on Post Harvested Maturity and Fruit Decay on Tomato**

The tomatoes were grown under either standard conditions (identified in Example 8) or full Messenger<sup>®</sup> treatment over the course of the growing season (identified in Example 8) and then hand picked at the time of commercial harvest. Mature green fruit of uniform size (5/6) were collected throughout the field in four replicate samples of 25 fruit per sample, placed directly into fruit bags and transported to a laboratory facility for postharvest treatment and/or analysis. Four different treatment regimen were examined as follows:

- (1) Fruits from Messenger<sup>®</sup> treated plots received no additional treatment following harvest;
- (2) Fruits from Messenger<sup>®</sup> treated plots were further treated with Messenger<sup>®</sup> after harvest;
- (3) Fruits from standard plots were treated with Messenger<sup>®</sup> after harvest; and
- (4) Fruits from standard plots received no additional treatment following harvest.

Postharvest treatment of fruits from groups (2) and (3) were carried out by dipping the fruit in a Messenger<sup>®</sup> solution (20 ppm harpin<sub>Ea</sub>). The postharvest treated tomatoes were allowed to air dry and then tomatoes from groups (1)-(4) were marked and mixed together in tomato crates for storage. Storage temperatures ranged from about 23 to 26°C (75-80°F). The tomatoes were then rated for color development and decay over time using the commercial rating scale from the Florida Tomato Committee color guide as follows:

**Grade Description**

- |   |  |
|---|--|
| 1 | Green: When fruit cut in half, no seeds cut; fruit entirely green with no color break;                   |
| 2 | Breakers: Initial sign of color break on 10% or less of the area of fruit; these areas are usually pink; |
| 3 | Turning: Pink or red on 10 to 30% of the fruit surface;  |

- 4 Pink: Pink or red on 30 to 60% of the fruit surface;
- 5 Light Red: Pink on over 60% of fruit surface and red color no more than 90% of fruit surface;
- 6 Red: Fruit totally red in color; and
- 5 7 Decay: Some areas of the fruit beginning to break down from decay.

The results of this treatment are set forth in Table 11 below.

Table 11: Affect of Preharvest and Postharvest Treatment on Maturity and Decay Data

Table 11: Effect of Preharvest and Postharvest Treatment on Quality and Shelf Life													
Group	Days After Treatment	Grade								Index	Efficacy	T-test	
		1	2	3	4	5	6	7	p<0.05			p<0.01	
1	3	80	18	2	0	0	0	0	0.17	0.00%	no	no	
2	3	73	17	9	1	0	0	0	0.20	-13.11%	yes	yes	
3	3	78	19	3	0	0	0	0	0.18	-2.46%	yes	yes	
4	3	80	18	2	0	0	0	0	0.17	N/A	no	no	
1	7	36	23	22	12	5	2	0	0.33	3.72%	yes	no	
2	7	37	23	17	19	4	0	0	0.33	4.96%	yes	no	
3	7	40	17	15	18	9	1	0	0.35	0.00%	yes	no	
4	7	35	22	19	15	8	1	0	0.35	N/A	no	no	
1	14	2	5	8	8	13	65	0	0.74	8.02%	yes	yes	
2	14	2	3	5	9	8	72	1	0.77	4.44%	yes	yes	
3	14	4	4	7	8	17	60	0	0.73	9.41%	yes	yes	
4	14	0	0	6	5	13	72	4	0.80	N/A	no	no	
1	17	0	0	2	3	6	89	0	0.83	2.51%	yes	yes	
2	17	1	1	1	0	7	88	2	0.83	2.35%	yes	yes	
3	17	1	2	0	0	9	88	0	0.83	3.18%	yes	yes	
4	17	0	0	0	0	7	89	4	0.85	N/A	no	no	
1	21	0	0	0	0	0	97	3	0.86	1.31%	yes	yes	
2	21	0	0	0	0	0	97	3	10.86	1.31%	yes	yes	
3	21	0	0	0	0	3	95	2	0.86	1.96%	yes	yes	
4	21	0	0	0	0	1	87	12	0.87	N/A	no	no	
1	28	0	0	0	0	0	85	15	0.88	2.84%	yes	yes	
2	28	0	0	0	0	0	91	9	0.87	3.79%	yes	yes	
3	28	0	0	0	0	0	81	19	0.88	2.21%	yes	yes	
4	28	0	0	0	0	0	67	33	0.90	N/A	no	no	
1	32	0	0	0	0	0	22	78	0.97	2.16%	yes	yes	
2	32	0	0	0	0	0	16	84	0.98	1.30%	yes	yes	
3	32	0	0	0	0	0	55	45	0.92	6.93%	yes	yes	
4	32	0	0	0	0	0	7	93	0.99	N/A	no	no	

Table 11 Cont.

Group	Days After Treatment	Grade							Index	Efficacy	T-test	
		1	2	3	4	5	6	7			p<0.05	p<0.01
1	37	0	0	0	0	0	14	86	0.98	1.15%	yes	yes
2	37	0	0	0	0	0	7	93	0.00	0.14%	yes	yes
3	37	0	0	0	0	0	9	91	0.99	0.43%	yes	yes
4	37	0	0	0	0	0	6	94	0.99	N/A	no	no
1	42	0	0	0	0	0	12	88	0.98	1.01%	yes	yes
2	42	0	0	0	0	0	7	93	0.99	0.29%	yes	yes
3	42	0	0	0	0	0	8	92	0.99	0.43%	yes	yes
4	42	0	0	0	0	0	5	95	0.99	N/A	no	no
1	45	0	0	0	0	0	8	92	0.99	0.57%	no	no
2	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	45	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	45	0	0	0	0	0	4	96	0.99	N/A	no	no
1	50	0	0	0	0	0	7	93	0.99	0.43%	no	no
2	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
3	50	0	0	0	0	0	4	96	0.99	0.00%	no	no
4	50	0	0	0	0	0	4	96	0.99	N/A	no	no

In previous trials tomatoes treated with Messenger<sup>®</sup> in the field and/or with a post harvest dip appeared to develop to red ripe more quickly, but decayed slower than the grower's standard. The data generated from this trial support these observations. By twenty-one days post harvest, 97% of the Messenger<sup>®</sup> treated tomatoes were full red ripe, compared to 87% of the grower's standard. Although it may be assumed that fruit which reach maturity more quickly will also start to break down more quickly, the results of the present Examples surprisingly demonstrate that these earlier-maturing tomatoes were actually 15% slower to decay than the grower's standard tomatoes. This phenomenon should be of great interest of several segments of the tomato market. The growers may be able to reduce ethylene gashouse timings, and the retail market should be able to significantly reduce inventory shrinkage.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

**WHAT IS CLAIMED:**

1. A method of inhibiting postharvest disease or desiccation in a fruit or vegetable, said method comprising:  
5 treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to inhibit postharvest disease or desiccation.
2. The method according to claim 1, wherein hypersensitive  
10 response elicitor protein or polypeptide is in isolated form.
3. The method according to claim 2, wherein said treating is carried out prior to harvest of the fruit or vegetable.
4. The method according to claim 3, wherein said treating is  
15 carried out by spraying the fruit or vegetable with the hypersensitive response elicitor protein or polypeptide.
5. The method according to claim 4, wherein the hypersensitive  
20 response elicitor protein or polypeptide is in liquid or powder form.
6. The method according to claim 1, wherein said treating is carried out after harvest of the fruit or vegetable.
7. The method according to claim 6, wherein said treating is  
25 carried out by spraying the fruit or vegetable with the hypersensitive response elicitor protein or polypeptide.
8. The method according to claim 7, wherein the hypersensitive  
30 response elicitor protein or polypeptide is in liquid or powder form.

9. The method according to claim 6, wherein said treating is carried out by immersing the fruit or vegetable in the hypersensitive response elicitor protein or polypeptide.

5 10. The method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

10 11. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

12. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

15 13. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

20 14. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

15. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

25 16. The method according to claim 10, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

17. The method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of *Phytophthora*.

30 18. The method according to claim 1, wherein said treating inhibits desiccation in a fruit or vegetable.

19. The method according to claim 1, wherein said treating inhibits a postharvest disease in a fruit or vegetable.

5 20. The method according to claim 19, wherein the postharvest disease is caused by *Penicillium*, *Botrytis*, *Phytophthora*, or *Erwinia*.

21. A method of inhibiting postharvest disease or desiccation in a fruit or vegetable, said method comprising:  
providing a transgenic plant or plant seed transformed with a  
10 DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and  
growing the transgenic plant or transgenic plant produced from  
the transgenic plant seed under conditions effective to inhibit a postharvest disease or  
desiccation in a fruit or vegetable harvested from the transgenic plant.

15 22. The method according to claim 21, wherein a transgenic plant is provided.

23. The method according to claim 21, wherein a transgenic plant seed is provided.

20 24. The method according to claim 21, wherein the transgenic plant is a dicot or a monocot.

25 25. The method according to claim 21, further comprising:  
applying the hypersensitive response elicitor polypeptide or  
protein to the fruit or vegetable to inhibit postharvest disease or desiccation.

26. The method according to claim 25, wherein said applying is carried out prior to harvest of the fruit or vegetable.

30 27. The method according to claim 25, wherein said applying is carried out after harvest of the fruit or vegetable.

28. The method according to claim 21, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of pathogen selected from the group consisting of *Erwinia*, *Xanthomonas*, *Pseudomonas*, *Phytophthora*, and *Clavibacter*.

5

29. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*.

10

30. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia carotovora*.

31. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia stewartii*.

15

32. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia chrysanthemi*.

33. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae*.

20

34. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas solanacearum*.

25

35. The method according to claim 28, wherein the hypersensitive response elicitor protein or polypeptide is derived from a species of *Phytophthora*.

36. The method according to claim 21, wherein the postharvest disease is caused by *Penicillium*, *Botrytis*, *Phytophthora*, or *Erwinia*.



37. A DNA construct comprising:  
a DNA molecule encoding a hypersensitive response elicitor protein or polypeptide;  
a plant-expressible promoter operably coupled 5' to the DNA molecule, the promoter being effective to transcribe the DNA molecule in fruit or vegetable tissue; and  
a 3' regulatory region operably coupled to the DNA molecule, wherein expression of the DNA molecule in fruit or vegetable tissue imparts to a fruit or vegetable resistance against postharvest disease or desiccation.
38. An expression system comprising a vector into which is inserted a heterologous DNA construct according to claim 37.
39. A host cell comprising a heterologous DNA construct according to claim 37.
40. The host cell according to claim 39, wherein the host cell is a plant cell or a bacteria cell.
41. The host cell according to claim 40, wherein the bacteria cell is an *Agrobacterium* cell.
42. A transgenic plant comprising a heterologous DNA construct according to claim 37.
43. A method of enhancing the longevity of fruit or vegetable ripeness comprising:  
treating a fruit or vegetable with a hypersensitive response elicitor protein or polypeptide under conditions effective to enhance the longevity of fruit or vegetable ripeness.

44. The method according to claim 43, wherein hypersensitive response elicitor protein or polypeptide is in isolated form.

5 45. The method according to claim 43, wherein said treating is carried out prior to harvest of the fruit or vegetable.

46. The method according to claim 43, wherein said treating is carried out after harvest of the fruit or vegetable.

10 47. A method of enhancing the longevity of fruit or vegetable ripeness comprising:  
providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and  
growing the transgenic plant or transgenic plant produced from  
15 the transgenic plant seed under conditions effective to enhance the longevity of fruit or vegetable ripeness in a fruit or vegetable harvested from the transgenic plant.

48. The method according to claim 47, further comprising:  
applying the hypersensitive response elicitor polypeptide or  
20 protein to the fruit or vegetable to enhance the longevity of fruit or vegetable ripeness.

49. The method according to claim 48, wherein said applying is carried out prior to harvest of the fruit or vegetable.

25 50. The method according to claim 48, wherein said applying is carried out after harvest of the fruit or vegetable.

## SEQUENCE LISTING

<110> Eden Bioscience Corporation

<120> TREATMENT OF FRUITS OR VEGETABLES WITH HYPERSENSITIVE  
RESPONSE ELICITOR TO CONTROL POSTHARVEST DISEASE OR  
DESICCATION

<130> 21829/72

<140>

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<150> 60/198,359

<151> 2000-04-19

<160> 12

<170> PatentIn Ver. 2.1

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<211> 338

<212> PRT

<213> Erwinia chrysanthemi

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&lt;210&gt; 3

&lt;211&gt; 403

&lt;212&gt; PRT

<213> *Erwinia amylovora*

&lt;400&gt; 3

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser





1	5	10	15
Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln	20	25	30
Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn	35	40	45
Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met	50	55	60
Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu	65	70	75
Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu	85	90	95
Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr	100	105	110
Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro	115	120	125
Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser	130	135	140
Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln	145	150	155
Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly	165	170	175
Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu	180	185	190
Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly	195	200	205
Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly	210	215	220
Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu	225	230	235
Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln	245	250	255
Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln			



260						265						270					
Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe		
		275					280					285					
Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met		
	290					295					300						
Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro		
305					310					315					320		
Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser		
				325					330					335			
Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn		
			340					345					350				
Lys	Ala	Lys	Gly	Met	Ile	Lys	Arg	Pro	Met	Ala	Gly	Asp	Thr	Gly	Asn		
		355					360					365					
Gly	Asn	Leu	Gln	Ala	Arg	Gly	Ala	Gly	Gly	Ser	Ser	Leu	Gly	Ile	Asp		
	370					375					380						
Ala	Met	Met	Ala	Gly	Asp	Ala	Ile	Asn	Asn	Met	Ala	Leu	Gly	Lys	Leu		
385					390					395					400		
Gly	Ala	Ala															

<210> 4

<211> 1288

<212> DNA

<213> Erwinia amylovora

<400> 4

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atcggcggtg	cgggcggaaa	taacgggttg	ctgggtacca	gtcgcagaa	tgctgggttg	180
ggtggcaatt	ctgcactggg	gctgggcggc	ggtaatacaa	atgataccgt	caatcagctg	240
gctggcttac	tcaccggcat	gatgatgatg	atgagcatga	tgggcggttg	tgggctgatg	300
ggcgttggt	taggcggttg	cttaggtaat	ggcttgggtg	gctcaggttg	cctgggcgaa	360
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ccgatgcagc	agctgctgaa	gatgttcagc	gagataatgc	aaagcctgtt	tggtgatggg	600
caagatggca	cccagggcag	ttcctctggg	ggcaagcagc	cgaccgaagg	cgagcagaac	660
gcctataaaa	aaggagtcac	tgatgcgctg	tcgggcctga	tgggtaatgg	tctgagccag	720



```

ctccttgcca acgggggact gggaggtggt cagggcggtta atgctggcac gggctcttgac 780
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```

&lt;210&gt; 5

&lt;211&gt; 447

&lt;212&gt; PRT

<213> *Erwinia amylovora*

&lt;400&gt; 5

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Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
  1             5             10             15

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Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
      20             25             30

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```

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
      35             40             45

```

```

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
      50             55             60

```

```

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
      65             70             75             80

```

```

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
      85             90             95

```

```

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
      100            105            110

```

```

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
      115            120            125

```

```

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
      130            135            140

```

```

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
      145            150            155            160

```



Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser  
 165 170 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro  
 180 185 190

Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro  
 195 200 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro  
 210 215 220

Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly  
 225 230 235 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His  
 245 250 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln  
 260 265 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn  
 275 280 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val  
 290 295 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala  
 305 310 315 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr  
 325 330 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn  
 340 345 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp  
 355 360 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe  
 370 375 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser  
 385 390 395 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser  
 405 410 415





Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu  
 420 425 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu  
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<210> 6

<211> 1344

<212> DNA

<213> *Erwinia amylovora*

<400> 6

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```

<210> 7

<211> 341

<212> PRT

<213> *Pseudomonas syringae*

<400> 7

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 1 5 10 15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser



	20		25		30														
Ser	Lys	Ala	Leu	Gln	Glu	Val	Val	Val	Lys	Leu	Ala	Glu	Glu	Leu	Met				
	35						40					45							
Arg	Asn	Gly	Gln	Leu	Asp	Asp	Ser	Ser	Pro	Leu	Gly	Lys	Leu	Leu	Ala				
	50					55					60								
Lys	Ser	Met	Ala	Ala	Asp	Gly	Lys	Ala	Gly	Gly	Gly	Ile	Glu	Asp	Val				
	65				70					75					80				
Ile	Ala	Ala	Leu	Asp	Lys	Leu	Ile	His	Glu	Lys	Leu	Gly	Asp	Asn	Phe				
				85					90					95					
Gly	Ala	Ser	Ala	Asp	Ser	Ala	Ser	Gly	Thr	Gly	Gln	Gln	Asp	Leu	Met				
			100					105					110						
Thr	Gln	Val	Leu	Asn	Gly	Leu	Ala	Lys	Ser	Met	Leu	Asp	Asp	Leu	Leu				
	115						120					125							
Thr	Lys	Gln	Asp	Gly	Gly	Thr	Ser	Phe	Ser	Glu	Asp	Asp	Met	Pro	Met				
	130					135					140								
Leu	Asn	Lys	Ile	Ala	Gln	Phe	Met	Asp	Asp	Asn	Pro	Ala	Gln	Phe	Pro				
	145				150					155					160				
Lys	Pro	Asp	Ser	Gly	Ser	Trp	Val	Asn	Glu	Leu	Lys	Glu	Asp	Asn	Phe				
				165					170					175					
Leu	Asp	Gly	Asp	Glu	Thr	Ala	Ala	Phe	Arg	Ser	Ala	Leu	Asp	Ile	Ile				
		180						185					190						
Gly	Gln	Gln	Leu	Gly	Asn	Gln	Gln	Ser	Asp	Ala	Gly	Ser	Leu	Ala	Gly				
	195						200					205							
Thr	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ser	Phe	Ser	Asn	Asn	Ser	Ser				
	210					215					220								
Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser				
	225				230					235					240				
Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp				
				245				250						255					
Arg	Gly	Leu	Gln	Ser	Val	Leu	Ala	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Val				
	260							265					270						
Asn	Thr	Pro	Gln	Thr	Gly	Thr	Ser	Ala	Asn	Gly	Gly	Gln	Ser	Ala	Gln				



275                      280                      285  
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala  
 290                      295                      300  
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala  
 305                      310                      315                      320  
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg  
 325                      330                      335  
 Asn Gln Ala Ala Ala  
 340

<210> 8

<211> 1026

<212> DNA

<213> *Pseudomonas syringae*

<400> 8

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<210> 9

<211> 424

<212> PRT

<213> *Pseudomonas syringae*

<400> 9

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu



1	5	10	15
Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly	20	25	30
Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly	35	40	45
Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val	50	55	60
Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile	65	70	75
Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr	85	90	95
Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln	100	105	110
Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser	115	120	125
Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr	130	135	140
Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly	145	150	155
Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly	165	170	175
Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr	180	185	190
Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr	195	200	205
Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile	210	215	220
Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp	225	230	235
Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp	245	250	255
Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr			





260	265	270
Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val		
275	280	285
Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln		
290	295	300
Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala		
305	310	315
Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp		
325	330	335
Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe		
340	345	350
Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln		
355	360	365
Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly		
370	375	380
Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr		
385	390	395
Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln		
405	410	415
Ala Ser Thr Gln His Thr Glu Leu		
420		

&lt;210&gt; 10

&lt;211&gt; 1729

&lt;212&gt; DNA

&lt;213&gt; Pseudomonas syringae

&lt;400&gt; 10

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gagcatcggc atcacacccc ggccgcaaca gaccaccacg ccaactcgatt tttcggcgct 480
aagcggcaag agtcctcaac caaacacggt cggcgagcag aacactcagc aagcgatcga 540

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&lt;210&gt; 11

&lt;211&gt; 344

&lt;212&gt; PRT

&lt;213&gt; Pseudomonas solanacearum

&lt;400&gt; 11

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Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
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Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
      20              25              30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
      35              40              45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
      50              55              60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
      65              70              75              80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
      85              90              95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
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Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala  
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 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala  
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 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly  
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 180 185 190  
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 195 200 205  
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 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp  
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 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val  
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 340

&lt;210&gt; 12



&lt;211&gt; 1035

&lt;212&gt; DNA

<213> *Pseudomonas solanacearum*

&lt;400&gt; 12

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